REMARKS

Reconsideration of the rejections set forth in the Office action mailed September 20, 2004 is respectfully requested. Claims 1-24 are pending in the application.

I. Amendments

The specification has been amended to provide consistency of language and to correct a typographical error.

Claim 1 has been amended to incorporate the subject matter of claims 2 and 8, which are accordingly canceled. Claim 1 has also been amended to specify that the high mobility ion (step b) is present in a higher concentration than the charged sample components, as stated, for example, at page 10, lines 6-7 of the specification, and that the sample components become stacked "into a small volume by ITP" (step c), as described, for example, at page 9, lines 16-17 of the specification.

Dependent claims 3, 9, and 10, which do not further limit the subject matter of amended claim 1, are also canceled.

No new matter is added by any of the amendments.

II. Rejections under 35 U.S.C. §103

Claims 1-8 and 14-24 were rejected under 35 U.S.C. §103(a) as being unpatentable over Manz *et al.* (U.S. Patent No. 6,280,589) in view of Krivankova *et al.* (*J. Chrom. B* **689**:13-34 (1997)). The rejections are respectfully traversed in light of the following remarks.

A. The Invention

The applicant's invention, as embodied in independent claim 1, is directed to a method of electrophoretically injecting a sample containing multiple charged components into a microfluidic device, and electrophoretically separating the components. The method as claimed employs a two-electrode injection step and a two-electrode separation step, and combines this voltage control scheme with ITP stacking, by supplying in either the sample solution or the buffer electrolyte solution a high mobility ion which is present at a higher concentration than the

sample ions. This method produces detectable peaks of high sharpness and resolution, while maintaining high sample volume, thus producing high peak intensities as well.

As discussed further below, cited reference Manz *et al.* describes a "pushback" strategy (which is referred to in the applicants' specification as "pullback"), and cited reference Ramsey *et al.* describes a "pinch" injection strategy. Both of these schemes employ simultaneous control of at least three electrodes. They have been shown to increase sample resolution and signal-to-noise ratio, but often at the expense of signal intensity.

The applicants have found that the use of transient isotachophoretic (ITP) stacking, which can be implemented as recited in steps (a)-(c) of claim 1, employed in combination with a two-electrode injection and two-electrode separation strategy (i.e. sample and drain voltage on for injection, followed by upstream and downstream voltage on for separation), gave significantly better peak sharpness and intensity than a "pinch/pullback" injection scheme, when the latter was done either with or without such ITP stacking. This is illustrated in Fig. 5C, which superimposes Figs. 5A and 5B. As shown in the Figures, while the "pinch/pullback" strategy gives a sharp and well resolved signal peak, its intensity is greatly reduced relative to the "floating" strategy, presumably due to sample loss.

The top two traces in Fig. 6A (which are offset for clarity) compare the "pushback" strategy described in Manz (labeled "FL INJ, PB SEP"; no pinching, no stacking) with the method of the invention (labeled "FL + STACK"; i.e. two electrode injection and two electrode separation, with ITP stacking). These results show that the method of the invention clearly provides greater peak sharpness and much greater peak intensity, with near-equivalent resolution (i.e. peak separation).

B. The Cited Art

Manz *et al.* describes, in the Background of the Invention, microfluidic sample injection processes in which sample is introduced electrophoretically into a main channel region by applying an appropriate voltage to each of two channels intersecting the main channel, one of which contains sample. Subsequently, the sample is separated by electrophoresis in the main channel. A perceived problem with this process, as described by Manz, is leakage of residual sample from these side channel(s) into the separation channel after sample introduction is

formally completed. Such leakage degrades resolution and signal-to-noise ratio of separated sample components at the detector. See, for example, the discussion in the Background of Manz *et al.* at column 1, lines 51-67.

To address this problem, Manz *et al.* employs a "pushback" strategy, where flow is induced back into the side channels to prevent the above-described leakage (see column 3, lines 1-10 and column 5, line 59 to column 6, line 5 of Manz *et al.*).

This injection strategy has been shown to improve resolution and signal-to-noise ratio; however, a common disadvantage of this injection strategy is loss of sample volume. This is shown, for example, in Figs. 5 and 6 of the applicants' specification, as described above.

The Examiner states (page 4 of Office Action) that "Manz *et al.* disclose the advantages of sample stacking within a prior art electrophoresis method (Column 1, lines 48-51)". However, the applicants find no reference to ITP or sample stacking in Manz *et al.* The reference makes no mention of the composition of the electrolyte buffer used for separation, and there is no reference to leading (high mobility) or trailing (low mobility) ions. The passage cited by the Examiner reads as follows:

> An advantage of the double T shape sampling device, as is also obtained with the use of injection valves, is the concentration effect of dilute sample ionic species. However, it is possible that, allthough [sic] no electric field gradient over the feeders exists, sample components from the feeders may diffuse into the capillary tube when the sample has already left the sampling position. The amounts of sample components that uncontrollably enter the capillary tube...

Based on the above passage, one can only conclude that Manz *et al.* attributes the "concentration effect" to "the double T shape sampling device". Moreover, the authors immediately proceed to a discussion of the diffusion (leakage) problem that can occur when "no electric field gradient over the feeders exists". Therefore, one must assume that this "concentration effect", however it is achieved, does nothing to address this diffusion problem.

Krivankova *et al.* describes the phenomenon of "sample induced stacking" or "sample induced transient ITP" at pages 28-31. While the authors note the advantage of "the adjustment

of concentration in the transient ITP step", they caution that "sample-induced stacking also brings a risk, that the zones may reach the detector earlier than they are unstacked" (e.g. as illustrated in Fig 23c) (page 31, first and second columns), and they state that the "conditions for the sample induced stacking being effective are very complex" (page 29, second column). Therefore, this reference does not teach that this method enhances the simplicity of a separation process.

C. Analysis

In view of the above comments, the applicants do not agree that it would have been obvious "to modify the method of Manz *et al.*" by using compositions that would provide sample stacking upon injection, as taught in Krivankova *et al.*, "because Manz *et al.* described such stacking as advantageous" (Office Action, pages 6-7).

Firstly, one skilled in the art would understand "the method of Manz *et al.*" to be the "pushback" injection strategy, since the "floating" injection strategy, used in the applicants' claimed method, was described in the Background of Manz *et al.* as problematic. Therefore, modifying "the method of Manz *et al.*" would not lead to the applicants' invention.

Secondly, as discussed above, Manz does not suggest the advantages of "stacking", and, while Krivankova *et al.* note the advantage of sample concentration in "sample induced stacking", they also note the risks of poor resolution and the complexity of the separation conditions.

(The Examiner also suggests that "a degree of sample stacking" would occur in the system of Manz *et al.* However, there is nothing in Manz *et al.* to suggest that the components would stack into a small volume "by ITP", as presently claimed.)

Finally, there is no suggestion in either reference that the use of ITP stacking in a two-electrode ("floating") injection/separation scheme would address the "leakage" problem noted by Manz *et al.*, by providing high peak sharpness and good resolution, without the concomitant loss of signal intensity shown by the Manz and Ramsey methods ("pushback" and "pinch", respectively).

"One way for a patent applicant to rebut a prima facie case of obviousness is to make a showing of 'unexpected results,' i.e. to show that the claimed invention exhibits some superior

property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected." *In re Soni*, 54 F.3d 746, 34 USPQ2d 1684 (Fed. Cir. 1995).

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

III. Further Rejections under 35 U.S.C. §103(a)

Dependent claims 9-12 were rejected under 35 U.S.C. §103(a) as being unpatentable over Manz *et al.* (U.S. Patent No. 6,280,589) in view of Krivankova *et al.* (*J. Chrom. B* **689**:13-34 (1997)), as applied to parent claim 1 above, and further in view of Ramsey (U.S. Patent No. 6,342,142). The rejections are respectfully traversed in light of the following remarks.

As noted above, claims 9-10 have been canceled. Dependent claims 11-12 are directed to types of analytes that may be used in the method of claim 1.

For the reasons discussed above, Manz *et al.* in view of Krivankova *et al.* does not teach or suggest the method of claim 1, or the benefits thereof. With respect to the subject matter of independent claim 1, Ramsey *et al.* does not make up for the deficiencies of these two references. For example, at column 5, line to 52 to column 6, line 13, Ramsey *et al.* describes the benefits of "pinched" injection over "floating" injection. The reference does not suggest the approach taken by the applicants, where ITP stacking is used in combination with "floating" injection and separation, and provides superior results to the "pinch/pullback" combination.

Accordingly, claim 1 and its dependent claims are nonobvious over this combination of references.

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

IV. Further Rejections under 35 U.S.C. §103(a)

Claims 1 and 13 were rejected under 35 U.S.C. §103(a) as being unpatentable over Fuchs *et al.* (U.S. Patent No. 5,630,924) in view of Manz *et al.* and Krivankova *et al.*, as presented above. The rejections are respectfully traversed in light of the following remarks.

A. The Cited Art

Manz *et al.* and Krivankova *et al.* are discussed above.

Fuchs *et al.* is directed to an assay method in which an analyte binds with a first binding partner, which is labeled, and with a second binding partner, which is modified to impart a significant charge. The binding partners are typically modified antibodies. The method is stated to improve over previous assays which did not employ the second binding partner, and where electrophoretic separation of unbound analyte from bound complex was less effective. (See, for example, column 1, lines 45-59 and column 2, lines 44-58.)

While there is a general description of electrophoretic mobility of species in Fuchs (column 16, lines 15-52), there is no description of particular voltage-controlled injection schemes, and, in fact, injection may be carried out using a pump rather than by voltage control (column 21, lines 26-38).

One stage of the assay in Fuchs involves mixing of the analyte and binding partners. One way of mixing these components is noted at column 23, lines 51-53, where "the elements of the mixture were concentrated in an electric field using a technique such as isoelectric focusing or isotachophoresis". There is no other reference to ITP in the patent.

B. Analysis

The Examiner suggests (page 12 of Office Action) that it would have been obvious to use the "sample inducing stacking" phenomenon described in Krivankova *et al.* to produce the ITP concentration effect in Fuchs, because "it requires a simpler electrolyte system than typical isotachophoretic methods". However, the Krivankova reference itself stated that the "conditions for the sample induced stacking being effective are very complex" (page 29, second column). Therefore, this reference does not teach that this method enhances the simplicity of a separation process.

The Examiner also stated that it would have been obvious to "allow the electrodes in the sample and waste reservoirs to float after injection, as taught by Manz *et al.*" However, both Manz *et al.* and Ramsey *et al.* describe the disadvantages of this injection system, as discussed above.

Finally, Fuchs *et al.* does not suggest the advantages of ITP in a separation method. As stated above, the only reference to ITP in Fuchs is as one way of mixing the assay components:

"the elements of the mixture were concentrated in an electric field using a technique such as isoelectric focusing or isotachophoresis". Presumably, the assay components are "concentrated" to facilitate reaction (binding) before the separation and detection of bound complex is carried out. Mixing of the components is further described at column 14, line 34-41:

> In one embodiment, first binding partner and second binding partner are combined with a sample to produce a mixture in which, if analyte is present, a three-membered complex forms. As used herein, the term "combine" is intended to mean any process by which multiple components are brought together for subsequent interaction at the molecular level.

The sole teaching of this reference with respect to ITP, then, is that it can be can be used as one way to combine assay components in a channel in high concentration, to facilitate reaction of the components with each other. This would not be perceived as a useful benefit per se in a process in which the ultimate goal is to separate all of the components from each other. There is no suggestion that the use of ITP stacking would produce any particular advantage with respect to separation.

In particular, none of the references, alone or in combination, suggest the benefits of ITP stacking discovered by the applicant, where the use of sample stacking in combination with a simple two-electrode injection scheme produced separation results superior to the "pullback" and "pinching" strategies described in the prior art.

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

V. Obviousness-Type Double Patenting Rejection

Claims 1-8 and 14-24 were rejected under the judicially created doctrine of obviousness-type double patenting as being directed to an invention not patentably distinct from claim 6 of commonly assigned U.S. Patent No. 6,818,113 (which issued from USSN 09/780,638).

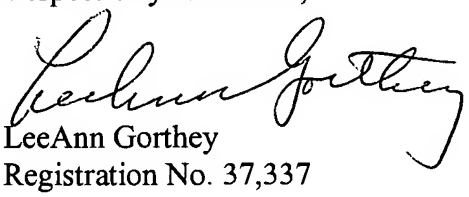
A Terminal Disclaimer prepared in accordance with 37 CFR §1.321(b) and (c) is enclosed. The signed Terminal Disclaimer will obviate the above obviousness-type double patenting rejection.

VI. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4403.

Respectfully submitted,



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